Cancer Incidence Among Pesticide Applicators Exposed to Chlorpyrifos in the Agricultural Health Study

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Background: **Chlorpyrifos is one of the most widely used insecticides in the United States. We evaluated the incidence of cancer among pesticide applicators exposed to chlorpyrifos in the Agricultural Health Study, a prospective cohort study of licensed pesticide applicators in Iowa and North Carolina.** ***Methods:*** **A total of 54 383 pesticide applicators were included in this analysis. Detailed information on pesticide exposure and lifestyle factors was obtained from self-administered questionnaires completed at the time of enrollment (December 1993–December 1997). Poisson regression analysis was used to evaluate the association between chlorpyrifos exposure and cancer incidence after adjustment for potential confounders. All statistical tests were two-sided.** ***Results:*** **A total of 2070 incident malignant neoplasms were diagnosed through 2001. The rate ratio for all cancers combined among chlorpyrifos-exposed applicators compared with nonexposed applicators was 0.97 (95% confidence interval = 0.87 to 1.08). For most cancers analyzed, there was no evidence of an exposure–response relationship. However, the incidence of lung cancer was statistically significantly associated with both chlorpyrifos lifetime exposure-days (P_{trend} = .002) and chlorpyrifos intensity-weighted exposure-days (P_{trend} = .036). After adjustment for other pesticide exposures and demographic factors, individuals in the highest quartile of chlorpyrifos lifetime exposure-days (>56 days) had a relative risk of lung cancer 2.18 (95% confidence interval = 1.31 to 3.64) times that of those with no chlorpyrifos exposure.** ***Conclusion:*** **Our findings suggest an association between chlorpyrifos use and incidence of lung cancer that deserves further evaluation. [J Natl Cancer Inst 2004;96:1781–9]**

Chlorpyrifos [*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)-phosphorothioate] is one of the most widely used organophosphate insecticides in the United States. According to the U.S. Environmental Protection Agency, chlorpyrifos, which has broad-spectrum insect toxicity, had an annual usage of 8–10 million pounds in the agricultural sector in 1999 *(1).* Approximately 800 registered products on the market contain chlorpyrifos, and these products are used for a number of purposes, including pest control for a variety of food crops, turf and ornamental plants, greenhouses, and sod; indoor pest control; structural pest control; and pet collars *(2).* Chlorpyrifos was widely used in U.S. households until 2000, when the U.S. Environmental Protection Agency revised its risk assessment of this and other organophosphate pesticides and phased out or eliminated certain residential uses *(3).* The primary urinary metabolite of chlorpyrifos (3,5,6-trichloro-2-pyridinol) has been detected in most of the subjects included in the population-based National Health and Nutrition Examination Survey III *(4)* and in 93% of 102 children in the Minnesota Children's Exposure Study in 2001 *(5).*

Chlorpyrifos is metabolized in the human liver to the active metabolite, chlorpyrifos oxon, which produces neurotoxicity by inhibiting esterases in the peripheral and central nervous system *(6).* There is little epidemiologic evidence of an association between chlorpyrifos exposure and human cancer, and most experimental studies have provided little or no evidence that chlorpyrifos has mutagenic or carcinogenic effects in humans *(2).* However, some experimental studies have detected chlorpyrifos-induced mutagenicity *(7,8)*, sister-chromatid exchanges *(9,10),* and chromosomal loss *(11).* In rats, chlorpyrifos has been found to induce mitotic abnormalities and cytotoxicity *(12)*; immunologic abnormalities, such as increased expression of the CD5 and CD8 surface markers *(13)*; and generation of reactive oxygen species, DNA damage, and lactate acid dehydrogenase leakage *(14).* In humans exposed to chlorpyrifos, expression of CD26 and the frequency of autoantibodies both increased *(15,16).* In addition, chlorpyrifos modifies endogenous antioxidants (i.e., superoxide dismutase, glutathione peroxidase, and glutathione) in rats, possibly leading to the development of oxidative stress *(17).* A case–control study reported increased risk of non-Hodgkin lymphoma among male farmers exposed to chlorpyrifos in the United States, although that result was based on only seven cases *(18).*

The Agricultural Health Study is a prospective cohort study *(19)* that was designed to examine a wide variety of occupational exposures among farmers and commercial pesticide applicators and the risk of cancer and other chronic diseases. The overall approach is to evaluate risk factors for specific diseases of interest, once sufficient numbers of exposed cases have been observed in the cohort [e.g., prostate cancer *(20)*], and also to evaluate disease risks among selected groups with specific exposures *(21).* The extensive use of chlorpyrifos in agriculture and in households in the United States and worldwide, its possible genotoxicity, and the lack of adequate epidemiologic

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information on cancer in chlorpyrifos-exposed populations prompted us to investigate cancer incidence among pesticide applicators exposed to chlorpyrifos who were enrolled in the Agricultural Health Study *(19)*.

Materials and Methods

Cohort Enrollment and Follow-up

The Agricultural Health Study is a prospective cohort study of certified pesticide applicators and their spouses in Iowa and North Carolina *(19)*. Recruitment began in December 1993 and continued through December 1997. A total of 57 311 pesticide applicators (82.4% of eligible applicators in both states) enrolled in the study by completing an enrollment questionnaire when they sought a restricted-use pesticide license from the state Cooperative Extension Services or Departments of Agriculture. Cohort members are matched to cancer registry files in Iowa and North Carolina annually for case identification and to the state death registries and the National Death Index annually to ascertain vital status. For the current analysis, incident cancers were identified from the date of enrollment (i.e., 1993–1997) through December 31, 2001. Cancers were coded according to the International Classification of Diseases for Oncology, 2nd edition (ICD-O-2) *(22)*. Cohort members who were alive at the end of follow-up but no longer residing in Iowa or North Carolina (n = 955) were identified through the current address records of the Internal Revenue Service, motor vehicle registration offices, and pesticide license registries of state agriculture departments and were censored in the year that they left the state. The average duration of follow-up was 6.4 years. All participants provided verbal informed consent, and institutional review boards of the National Cancer Institute, Battelle (the field station in North Carolina), the University of Iowa (the field station in Iowa), and Westat (coordinating center for the study) approved the protocol.

Exposure Assessment

The self-administered enrollment questionnaire collected comprehensive exposure data on 22 pesticides and ever/never use information on 28 additional pesticides, as well as information on use of personal protective equipment, pesticide application methods, pesticide mixing status, equipment repair methods, smoking history, alcohol consumption, history of cancer in first-degree relatives, and basic demographic data. All participants who completed the enrollment questionnaire were also given a self-administered take-home questionnaire that included more detailed questions about occupational history and questions on medical history and diet. Both questionnaires may be found at http://www.aghealth.org/questionnaires.html. A total of 24 671 pesticide applicators (43%) returned the take-home questionnaire. Participants who did and did not return the take-home questionnaire were similar with regard to farming practices, medical history including previous lung diseases, and demographic characteristics, except age distribution *(23)*.

Data from the enrollment questionnaire and measurement data from the published pesticide exposure literature were used to estimate the intensity of exposure to individual pesticides using the following formula: intensity level = (mixing status + application method + equipment repair status) × personal protective equipment use, where the various levels of the four elements of the intensity score were weighted to reflect their importance to exposure *(24)*. Mixing status was a three-level variable, based on never mixing, mixing less than 50% of the time, and mixing at least 50% of the time (values of 0, 3, and 9, respectively). Application method was a six-level variable, based on never applying, use of aerial-aircraft or distribution of tablets, application in furrow, use of boom on tractor, use of backpack, and use of hand spray (values of 0, 1, 2, 3, 8, and 9, respectively). Equipment repair status was a two-level variable, based on not repairing and repairing pesticide application equipment (0 and 2, respectively). Personal protective equipment use was coded as an eight-level variable based on the percentage of time that personal protective equipment was used while applying pesticides.

We constructed two lifetime chlorpyrifos exposure variables for this analysis, each categorized into quartiles based on the

Table 1. Selected characteristics of applicators by chlorpyrifos exposure based on 1993–1997 enrollment data in the Agricultural Health Study*

Characteristics	Exposed, No. (%) (n = 22 181)	Nonexposed, No. (%) (n = 32 202)
Age, y		
<40	7297 (32.9)	10 473 (32.5)
40–49	6715 (30.3)	8388 (26.0)
50–59	4600 (20.7)	6584 (20.5)
≥60	3569 (16.1)	6755 (21.0)
Sex		
Male	21 859 (98.5)	31 051 (96.4)
Female	322 (1.5)	1151 (3.6)
State of residence		
Iowa	14 842 (66.9)	20 668 (64.2)
North Carolina	7339 (33.1)	11 534 (35.8)
Applicator type†		
Private	20 674 (93.2)	28 909 (89.8)
Commercial	1507 (6.8)	3293 (10.2)
Smoking history‡		
Never	11 836 (54.9)	16 807 (54.1)
Low (<12 pack-years)	4940 (22.9)	6980 (22.4)
High (≥12 pack-years)	4781 (22.2)	7290 (23.5)
Alcohol consumption§		
No	6372 (29.7)	10 393 (33.9)
Yes	15 093 (70.3)	20 277 (66.1)
Educational level		
High school or less	11 841 (54.6)	18 503 (58.9)
Beyond high school	9852 (45.4)	12 897 (41.1)
Family history of cancer in first-degree relative		
No	11 785 (57.7)	17 832 (61.9)
Yes	8649 (42.3)	10 986 (38.1)
Use of pesticides most highly correlated with use of chlorpyrifos		
Alachlor	13 481 (64.4)	12 806 (43.3)
Carbofuran	7764 (37.6)	5257 (17.8)
Fonofos	6079 (29.3)	4314 (14.5)
Trifluralin	12 414 (59.3)	13 899 (46.8)

*Information on smoking history, alcohol consumption, educational level, family history of cancer, and use of the four pesticides whose use is most highly correlated with use of chlorpyrifos (alachlor, carbofuran, fonofos, trifluralin) were missing for 5.6%, 6.4%, 4.4%, 11.6%, and 9.6% of participants, respectively.

†The term "private" refers primarily to individual farmers, and "commercial" refers to professional pesticide applicators.

‡The cut point between low and high was set at the median level of pack-years among smokers.

§Based on the answer to the question "During the past 12 months, how often did you drink any kind of alcoholic beverage?"

Table 2. Rate ratios (RRs) and 95% confidence intervals (CIs) for selected cancers by chlorpyrifos exposure status of the Agricultural Health Study applicators, 1993–2001*

Cause of cancer (ICD-O-2)	All enrolled applicators (n = 54 383)			Applicators who completed take-home questionnaires (n = 24 671)		
	Exposed	Nonexposed	RR (95% CI)†	Exposed	Nonexposed	RR (95% CI)‡
All malignant neoplasms	765	1305	0.97 (0.87 to 1.08)	403	708	0.99 (0.86 to 1.15)
Buccal cavity, pharynx	19	38	1.05 (0.56 to 1.94)	8	23	0.75 (0.30 to 1.86)
Esophagus	14	10	1.88 (0.69 to 5.13)	10	7	2.28 (0.72 to 7.17)
Stomach	11	15	1.09 (0.40 to 2.92)	5	12	0.72 (0.20 to 2.62)
Colorectal	92	157	0.87 (0.63 to 1.21)	45	85	0.92 (0.59 to 1.44)
Colon	57	116	0.72 (0.48 to 1.07)	29	66	0.75 (0.44 to 1.28)
Rectum	35	41	1.33 (0.75 to 2.36)	16	19	1.58 (0.69 to 3.58)
Pancreas	10	27	0.36 (0.13 to 0.97)	6	14	0.62 (0.19 to 2.01)
Lung	75	126	1.36 (0.96 to 1.93)	41	61	1.49 (0.94 to 2.38)
Melanoma	34	41	1.11 (0.65 to 1.88)	18	26	1.04 (0.52 to 2.07)
Prostate	297	523	0.91 (0.76 to 1.09)	168	297	0.95 (0.75 to 1.19)
Bladder	24	54	0.75 (0.43 to 1.31)	13	25	1.02 (0.50 to 2.06)
Kidney	20	41	1.08 (0.56 to 2.06)	13	17	2.07 (0.89 to 4.77)
Brain	15	13	1.77 (0.70 to 4.50)	4	7	1.25 (0.31 to 4.99)
All lymphohematopoietic cancers	75	114	1.02 (0.73 to 1.44)	40	56	1.06 (0.66 to 1.68)
Non-Hodgkin lymphoma	37	53	1.03 (0.62 to 1.70)	19	21	1.26 (0.61 to 2.62)
Multiple myeloma	10	22	0.77 (0.32 to 1.82)	6	13	0.66 (0.23 to 1.94)
Leukemia	23	34	1.05 (0.57 to 1.93)	13	20	1.06 (0.48 to 2.34)

*Cancer subtypes with fewer than 10 exposed cases are not shown. ICD-O-2 = International Classification of Diseases for Oncology, 2nd edition.

†Rate ratio adjusted for age, sex, alcohol consumption, smoking history, educational level, family history of cancer, year of enrollment, state of residence, and use of the four pesticides whose use is most highly correlated with use of chlorpyrifos (alachlor, carbofuran, fonofos, trifluralin) among all enrollment applicators. The reference category was applicators who were not exposed to chlorpyrifos.

‡Rate ratio was adjusted for age, sex, alcohol consumption, smoking history, educational level, family history of cancer, year of enrollment, state of residence, use of the four pesticides whose use is most highly correlated with use of chlorpyrifos (alachlor, carbofuran, fonofos, trifluralin), and other occupational exposures (asbestos, engine exhaust, silica/sand dust, welding fumes) among applicators who completed the take-home questionnaire.

distribution of all cancer cases among chlorpyrifos-exposed applicators. The first, lifetime exposure-days, was obtained by multiplying the midpoints of the questionnaire categories of number of years an applicator personally applied or mixed chlorpyrifos by the number of days in an average year an applicator personally mixed or applied chlorpyrifos (i.e., years of use × days used per year, resulting in the following quartiles: ≤8.8, 8.9–24.5, 24.6–56.0, ≥56.1). The second lifetime chlorpyrifos exposure variable, intensity-weighted exposure-days, was obtained by multiplying the midpoint of lifetime exposure-days by the midpoint of intensity level (i.e., years of use × days used per year × intensity level, resulting in the following quartiles: ≤48.9, 49.0–135.9, 136.0–417.6, ≥417.7).

Statistical Analysis

Participants with prevalent cancer at enrollment (n = 1074) or who did not provide information on chlorpyrifos exposure (n = 1854) were excluded from this analysis, leaving 22 181 chlorpyrifos-exposed and 32 202 nonexposed applicators. Of those excluded, 44% were from Iowa and 56% were from North Carolina. Those excluded were older than the rest of the cohort and were more likely than the rest of the cohort to have some other missing data. However, they were similar to the rest of the cohort on smoking and overall pesticide use (data not shown).

We used Poisson regression in the Stata program (version 8.0) *(25)* to examine exposure–response associations within the cohort and to explore the effect of potential confounding factors. Rate ratios (RRs) derived from the analysis were adjusted for age at enrollment (<40, 40–49, 50–59, ≥60 years), sex, educational level (high school graduate or less, beyond high school), smoking history (never/low/high, with smokers below the median value of 12 pack-years classified as low and smokers above the median classified as high), any alcohol consumption during the 12 months prior to enrollment (yes/no), history of cancer in a first-degree relative (yes/no), state of residence (Iowa/North Carolina), and year of enrollment. For pesticide category–specific rate ratios, we used applicators not exposed to chlorpyrifos as the reference category. Because of potential concomitant exposure to other pesticides, we adjusted rate ratios for exposure to the four pesticides (alachlor, carbofuran, fonofos, trifluralin) whose use was most highly correlated with intensity-weighted exposure-days to chlorpyrifos (Pearson correlation coefficient $r \geq .4$). The exposure levels of these four pesticides were categorized as never, low, and high; for each pesticide, the cut point between low and high exposure was set at the median of intensity-weighted exposure-days. We also added a variable for total years of pesticide application into the model as a surrogate measure of other potential farming exposure. We evaluated departures from a log linear model by adding a quadratic term for exposure-days and using a log likelihood test. We analyzed exposure–response trends by including the categorical score (i.e., 0, 1, 2, 3) of each quartile as a continuous variable in the model and testing for the statistical significance of the slope. All tests of statistical significance were two-sided.

We conducted further analyses for lung cancer by stratifying on state of residence, histologic type, smoking history, and the first decade of chlorpyrifos use to investigate the consistency of associations observed. We also used data on the 24 671 participants who completed the self-administered take-home questionnaire to calculate rate ratios for lung cancer among chlorpyrifos-exposed applicators. These participants had additional information for other potential confounding factors, including previous lung

Table 3. Rate ratios (RRs) and 95% confidence intervals (CIs) for selected cancers by lifetime chlorpyrifos exposure-days and intensity-weighted chlorpyrifos exposure-days among Agricultural Health Study applicators, 1993–2001*

	Lung cancer		Rectal cancer		Brain cancer		Esophageal cancer		Kidney cancer	
Pesticide exposure	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)
Lifetime chlorpyrifos exposure-days†										
Nonexposed	126	1.0 (referent)	41	1.0 (referent)	13	1.0 (referent)	10	1.0 (referent)	41	1.0 (referent)
0.1–8.8	14	0.77 (0.41 to 1.45)	10	1.09 (0.44 to 2.68)	3	1.22 (0.26 to 5.77)	4	2.21 (0.55 to 8.89)	7	1.27 (0.51 to 3.14)
8.9–24.5	19	1.63 (0.95 to 2.78)	5	0.90 (0.31 to 2.62)	2	0.68 (0.08 to 5.46)	4	2.16 (0.54 to 8.68)	2	0.57 (0.13 to 2.42)
24.6–56.0	16	1.44 (0.77 to 2.68)	4	0.28 (0.04 to 2.09)	4	3.18 (0.93 to 10.92)	3	2.25 (0.54 to 9.30)	4	1.03 (0.31 to 3.48)
≥56.1	24	2.18 (1.31 to 3.64)	15	3.25 (1.60 to 6.62)	6	2.58 (0.73 to 9.17)	2	0.52 (0.06 to 4.57)	7	1.49 (0.55 to 4.07)
P_{trend}		.002		.035		.076		.835		.665
Intensity-weighted chlorpyrifos exposure-days‡										
Nonexposed	126	1.0 (referent)	41	1.0 (referent)	13	1.0 (referent)	10	1.0 (referent)	41	1.0 (referent)
0.1–45.9	16	1.31 (0.70 to 2.43)	5	0.47 (0.11 to 2.03)	1	—	3	2.30 (0.45 to 11.80)	6	1.66 (0.62 to 4.44)
49.0–135.9	11	1.07 (0.55 to 2.09)	7	1.18 (0.44 to 3.16)	5	3.32 (0.98 to 11.24)	3	1.76 (0.35 to 8.92)	2	0.65 (0.15 to 2.7)
136.0–417.6	18	1.53 (0.86 to 2.73)	7	0.73 (0.22 to 2.46)	2	1.25 (0.26 to 6.10)	3	1.89 (0.45 to 7.86)	4	0.86 (0.25 to 2.94)
≥417.7	19	1.80 (1.00 to 3.23)	10	3.16 (1.42 to 7.03)	7	4.03 (1.18 to 13.79)	3	1.17 (0.21 to 6.35)	6	1.30 (0.42 to 4.00)
P_{trend}		.036		.057		.036		.549		.904

diseases (yes/no), vegetable intake (low, medium, high), type of farm (e.g., corn or tobacco), and exposure to occupational factors such as asbestos, engine exhaust, silica, and welding fumes (yes/no). We also attempted to examine multiplicative interactions among chlorpyrifos exposure, other occupational factors, and risk of lung cancer. Our primary analysis focused on first primary lung cancers. We repeated the analysis including both first and second primary lung cancers; the results were essentially unchanged.

Results

Table 1 shows selected characteristics of chlorpyrifos-exposed and nonexposed applicators. Among subjects with complete exposure information, 22 181 (41%) reported that they had ever used chlorpyrifos. The majority of the cohort consisted of male private applicators, and more than 60% of the cohort members were under age 50 years. Approximately two-thirds of the cohort members lived in Iowa, and more than 50% were never smokers. All variables in Table 1 showed only a small difference between chlorpyrifos-exposed and nonexposed applicators, except for the frequency of use of the four pesticides whose use is most highly correlated with the use of chlorpyrifos.

The risk of cancer associated with chlorpyrifos use among all applicators and among the subset who completed the take-home questionnaire (for whom we have data on additional potential confounding variables) is shown in Table 2. A total of 765 and 1305 incident cancers were observed among chlorpyrifos-exposed and nonexposed applicators, respectively. The corresponding numbers of cases in the subgroup that completed the take-home questionnaire were 403 and 708, respectively. For all cancers combined, the rate ratio was 0.97 (95% confidence interval [CI] = 0.87 to 1.08). Pancreatic cancer risk among chlorpyrifos-exposed applicators was lower than that among nonexposed applicators, but this finding was based on relatively small numbers of exposed patients and was not statistically significant after adjusting for the confounding factors (e.g., occupational exposure to asbestos, engine exhaust, silica, and welding fumes) addressed in the take-home questionnaires. Chlorpyrifos exposure was associated with increased risks of several cancers, including cancers of the esophagus, rectum, lung, kidney, and brain, although none of the increases was statistically significant. The increased risk of lung cancer seen after the initial adjustment (i.e., for other pesticide exposures and demographic factors) (RR = 1.36, 95% CI = 0.96 to 1.93) remained after adjusting for the confounding factors addressed in the take-home questionnaire (RR = 1.49, 95% CI = 0.94 to 2.38). Risk estimates for lung cancer were similar for state-specific analyses (data not shown). Risk estimates also did not differ when the lung cancer analysis was restricted to first primary cancers or when using all incident lung cancer cases, including 17 additional second primary lung cancers (data not shown).

Exposure–response relationships by chlorpyrifos lifetime exposure-days and lifetime intensity-weighted exposure-days were evaluated for cancers of the lung, rectum, brain, esophagus, kidney, and lymphohematopoietic system (Table 3) based on either the positive findings in Table 2 or because the scientific literature *(26)* suggests that exposure to agricultural chemicals may be associated with excess incidence of some of these cancers. We used these two measures of chlorpyrifos exposure because information on exposure-days was available for more study subjects, although we believe that intensity-weighted exposure-days may provide more accurate information on dermal exposure. Statistically significant trends in increasing lung cancer incidence were seen with both lifetime exposure-days (P_{trend} = .002) and intensity-weighted exposure-days (P_{trend} = .036), with the rate in the highest exposure category approximately twice that of the rate in the unexposed category. The lung cancer trend was not changed when we added "total years of pesticide application" to the multivariable analysis as a surrogate measure of other potential farming exposures or when we adjusted for smoking history using number of packs smoked per day or years smoked as separate variables (data not shown). Rectal cancer incidence also showed a statistically significant increase with increasing lifetime exposure-days to chlorpyrifos (P_{trend} = .035), although this trend was largely the result of the increased risk in the highest exposure category. No exposure–response trend was seen for colon cancer. Individuals in the highest category of intensity-weighted exposure-days but not lifetime exposure-days had statistically significant increases in

Table 3 (continued).

Pesticide exposure	All lymphohematopoietic cancers		Non-Hodgkin lymphoma		Leukemia		Multiple myeloma	
	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)
Lifetime chlorpyrifos exposure-days†								
Nonexposed	114	1.0 (referent)	53	1.0 (referent)	34	1.0 (referent)	22	1.0 (referent)
0.1–8.8	18	0.67 (0.36 to 1.24)	10	0.60 (0.23 to 1.54)	7	1.07 (0.43 to 2.65)	1	0.29 (0.04 to 2.24)
8.9–24.5	20	1.24 (0.74 to 2.09)	13	1.79 (0.92 to 3.48)	2	0.46 (0.11 to 1.96)	2	0.36 (0.05 to 2.78)
24.6–56.0	11	0.92 (0.49 to 1.75)	5	0.91 (0.35 to 2.35)	2	0.57 (0.13 to 2.41)	3	1.28 (0.35 to 4.60)
≥56.1	24	1.43 (0.86 to 2.36)	9	1.01 (0.43 to 2.35)	10	2.15 (0.96 to 4.81)	4	1.49 (0.46 to 4.85)
P_{trend}		0.261		0.725		0.356		0.643
Intensity-weighted chlorpyrifos exposure-days‡								
Nonexposed	114	1.0 (referent)	53	1.0 (referent)	34	1.0 (referent)	22	1.0 (referent)
0.1–48.9	11	0.71 (0.35 to 1.43)	6	0.85 (0.33 to 2.20)	4	0.76 (0.22 to 2.57)	0	—
49.0–135.9	10	0.57 (0.27 to 1.19)	6	0.62 (0.22 to 1.76)	3	0.70 (0.21 to 2.37)	1	0.34 (0.04 to 2.68)
136.0–417.6	18	1.06 (0.60 to 1.86)	10	1.24 (0.57 to 2.74)	2	0.48 (0.11 to 2.05)	4	1.03 (0.28 to 3.75)
≥417.7	25	1.99 (1.22 to 3.26)	10	1.61 (0.74 to 3.53)	10	3.01 (1.35 to 6.69)	3	1.24 (0.32 to 4.75)
P_{trend}		0.091		0.385		0.151		0.955

*Rate ratios were adjusted for age, sex, alcohol consumption, smoking history, educational level, family history of cancer, year of enrollment, state of residence, and use of the four pesticides whose use is most highly correlated with use of chlorpyrifos (alachlor, carbofuran, fonofos, trifluralin). (Applicators who did not provide information on chlorpyrifos days used per year, years of use, and intensity level were excluded from this analysis.)

†Lifetime exposure days = years of use × days used per year. Cut points based on the distribution of all cancer cases among chlorpyrifos-exposed applicators.

‡Intensity-weighted exposure days = years of use × days used per year × intensity level. Cut points based on the distribution of all cancer cases among chlorpyrifos-exposed applicators.

rates of all lymphohematopoietic cancers, leukemia, and brain cancer compared with nonexposed individuals. We did not observe an exposure–response pattern for any other cancer. We also repeated the analyses using the applicators in the lowest exposure category as the reference group to mitigate the possibility of residual confounding. The results of these analyses were similar to those in which nonexposed individuals were used as the reference group (data not shown). For example, the rate ratios for lung cancer were 2.16 (95% CI = 1.01 to 4.64), 1.86 (95% CI = 0.81 to 4.28), and 2.96 (95% CI = 1.38 to 6.37) for the second, third, and fourth exposure quartiles, respectively, relative to the first quartile. The risks were not changed when we used detailed categorical variables for alcohol consumption (i.e., never, less than one time a month, 1–3 times a month, 1 time a week, 2–4 times a week, almost every day, every day).

To further examine the chlorpyrifos–lung cancer association, we calculated rate ratios for lung cancer according to lifetime exposure-days to chlorpyrifos (Table 4) stratified by state of residence, histologic type, and smoking history. Lung cancer risks increased with chlorpyrifos exposure in both states and for all histologic types, although the trend was statistically significant in North Carolina and for adenocarcinoma only. The instability of these risk estimates is probably due to the relatively small number of exposed cases. A statistically significant exposure–response trend was also found among current smokers. However, we were unable to investigate risk trends among nonsmokers because of the small numbers of cases. Increased lung cancer risks with increasing chlorpyrifos exposure were also seen after stratification for previous lung diseases, including chronic bronchitis and pneumonia; for family history of lung cancer; for type of farm; or after adjusting for vegetable intake (data not shown). Stratified analysis by first decade of chlorpyrifos use showed increased risk with earlier decade of first use: Compared with nonexposed applicators, the rate ratios were 1.59 (95% CI = 0.91 to 2.78) among those who first used chlorpyrifos during the 1970s, 1.38 (95% CI = 0.90 to 2.11) among those who first used it during the 1980s, and 1.26 (95% CI = 0.68 to 2.33) among those who first used it during the 1990s.

We also examined lung cancer risks associated with combined exposure to chlorpyrifos and other agents that are potential risk factors for lung cancer (Table 5). In all cases, the rate ratios associated with combined exposure were higher than the rate ratios for each individual agent. However, no statistically significant interactions were observed. The interaction rate ratio for lung cancer with chlorpyrifos and smoking was limited to a comparison of current smokers versus nonsmokers (i.e., never and former smokers combined) because of the small number of never-smoking patients with lung cancer in the cohort at this time.

Discussion

In this analysis of cancer incidence among chlorpyrifos-exposed licensed pesticide applicators in North Carolina and Iowa, we found a statistically significant trend of increasing risk of lung cancer, but not of any other cancer examined, with increasing chlorpyrifos exposure. The lung cancer association was not explained by smoking, previous lung disease, other occupational exposures, or type of farm. The exposure–response trends were similar using two different referent groups—those never exposed to chlorpyrifos, and those in the lowest quartile of chlorpyrifos exposure. Individuals in the highest quartile of chlorpyrifos use (i.e., with more than 56 lifetime exposure-days) had twice the risk of lung cancer as applicators who never used chlorpyrifos, although this elevation in risk may be restricted to current smokers.

Most previous studies have shown lower lung cancer rates for farmers than for the general population, which are probably due to a lower prevalence of smoking among farmers *(27).* Although several studies have reported increased lung cancer risk among

Table 4. Rate ratios (RRs) and 95% confidence intervals (CIs) for lung cancer by lifetime chlorpyrifos exposure-days among Agricultural Health Study applicators stratified by state of residence, smoking history, and histologic types of lung cancer, 1993–2001*

	Lifetime chlorpyrifos exposure-days								
	0.1–8.8		8.9–24.5		24.6–56.0		≥56.1		
Variable	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	P_{trend}
State of residence									
Iowa	4	0.36 (0.09 to 1.54)	10	2.12 (1.02 to 4.41)	3	0.75 (0.22 to 2.46)	12	2.25 (1.06 to 4.79)	.063
North Carolina	10	0.98 (0.48 to 2.01)	9	1.20 (0.54 to 2.71)	13	2.02 (0.95 to 4.27)	12	2.12 (1.04 to 4.30)	.019
Smoking history†									
Current	6	0.97 (0.40 to 2.34)	8	1.40 (0.57 to 3.40)	10	2.33 (1.04 to 5.22)	14	3.11 (1.58 to 6.12)	.001
Former	6	0.40 (0.12 to 1.31)	10	1.71 (0.84 to 3.49)	6	0.75 (0.26 to 2.16)	9	1.14 (0.45 to 2.86)	.776
Never	2	2.70 (0.50 to 14.46)	1	1.92 (0.22 to 16.95)	0	—	1	2.24 (0.25 to 19.91)	.636
Histologic type‡									
Adenocarcinoma	4	1.24 (0.41 to 3.74)	6	1.95 (0.70 to 5.44)	3	1.36 (0.39 to 4.78)	10	3.13 (1.27 to 7.75)	.022
Squamous cell	6	1.64 (0.61 to 4.45)	7	3.33 (1.37 to 8.12)	3	1.24 (0.28 to 5.50)	5	2.00 (0.64 to 6.28)	.088
Epithelial	2	0.29 (0.07 to 1.21)	5	0.81 (0.29 to 2.32)	8	1.30 (0.49 to 3.40)	8	1.82 (0.81 to 4.09)	.257

*Rate ratios were adjusted for age, sex, alcohol consumption, smoking history, educational level, family history of cancer, year of enrollment, state of residence, and use of the four pesticides whose use is most highly correlated with use of chlorpyrifos (alachlor, carbofuran, fonofos, trifluralin). The reference category was applicators who were not exposed to chlorpyrifos. Lifetime exposure-days = years of use × days used per year. Cut points were based on the distribution of all cancer cases among chlorpyrifos-exposed applicators.

†Rate ratios for smoking status category were also adjusted for smoking by pack-years as a continuous variable.

‡Histologic types with fewer than five exposed cases are not shown.

pesticide-exposed populations, especially among those using DDT, diazinon, carbaryl, or propoxur *(28–30)*, the lack of information on tobacco use and on the details of specific pesticide use, limited causal interpretation. Overall, our cohort had a lower risk of lung cancer than the Iowa and North Carolina populations *(31)*, but we did detect an exposure–response pattern between chlorpyrifos exposure and lung cancer risk after controlling for other known cancer risk factors.

The mechanism by which chlorpyrifos increases lung cancer risk is not known. However, experimental studies suggest that chlorpyrifos may induce immune alteration *(13,15,16)* and oxidative stress *(14,17)* and may decrease the activity of glutathione *S*-transferase in animals and humans *(32,33)*. Because glutathione conjugation represents the major pathway for elimination of benzopyrene epoxides in the lung, this pathway may offer an explanation for the possible co-carcinogenicity of chlorpyrifos in combination with polycyclic aromatic hydrocarbons from exposures such as cigarette smoking. Studies in the amphipod *Hyalella azteca* have shown that co-exposure to chlorpyrifos and methyl mercury increases toxicity additively by competition for glutathione, which would decrease the elimination of chlorpyrifos *(32,34)*. Although this observation was made in an amphi-

Table 5. Rate ratios (RRs) and 95% confidence intervals (CIs) for lung cancer by co-exposure to chlorpyrifos and other occupational exposures among the Agricultural Health Study applicators who completed the take-home questionnaire, 1993–2001*

	Not exposed to chlorpyrifos (n = 10 644)		Exposed to chlorpyrifos (n = 12 032)		Interaction RR (95% CI)
Co-exposure	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	
Asbestos					
No	52	1.0 (referent)	35	1.42 (0.86 to 2.36)	
Yes	9	1.80 (0.80 to 4.07)	6	3.54 (1.47 to 8.51)	1.38 (0.41 to 4.58)
Engine exhaust					
No	47	1.0 (referent)	29	1.35 (0.79 to 2.30)	
Yes	14	1.06 (0.52 to 2.14)	12	2.10 (1.01 to 4.37)	1.47 (0.53 to 4.08)
Silica/sand dust					
No	58	1.0 (referent)	35	1.37 (0.84 to 2.24)	
Yes	3	1.75 (0.54 to 5.68)	6	5.11 (1.98 to 13.22)	2.13 (0.47 to 9.69)
Welding fumes					
No	51	1.0 (referent)	33	1.53 (0.92 to 2.55)	
Yes	10	1.32 (0.63 to 2.76)	8	1.69 (0.70 to 4.09)	0.84 (0.27 to 2.64)
Current cigarette smoking†					
No	73	1.0 (referent)	35	1.14 (0.72 to 1.80)	
Yes	52	4.48 (2.93 to 6.84)	38	7.75 (4.89 to 12.29)	1.42 (0.75 to 2.70)

*Rate ratios were adjusted for age, sex, alcohol consumption, smoking history, educational level, family history of cancer, year of enrollment, state of residence, and use of the four pesticides whose use is most highly correlated with use of chlorpyrifos (alachlor, carbofuran, fonofos, trifluralin). For each co-exposure, the reference group was the applicators in the not-exposed-to-chlorpyrifos group who were not exposed to that agent. Interaction RR was calculated by using a likelihood ratio test.

†Total number of applicators who completed the enrollment questionnaire. (Smoking information was available to all applicators who completed the enrollment questionnaire.)

pod, it does provide a link to glutathione, which may be important biologically. These reported biologic activities of chlorpyrifos may explain the enhanced effects of chlorpyrifos in combination with other exposures and may also account for the observed lung cancer risk, despite the lack of consistent evidence for carcinogenicity in animal studies. A more complete analysis of lung cancer risk and the interaction of chlorpyrifos and other exposures will be possible as additional cases occur in the Agricultural Health Study; these analyses will also be important in helping to explain the mechanism by which chlorpyrifos is associated with increased lung cancer risk.

A limitation of this analysis as it relates to lung cancer is the possible confounding effect of smoking. Because of the small number of lung cancer patients who had been exposed to chlorpyrifos, we could not analyze data separately for women ($n = 3$) or for nonsmokers ($n = 4$). The lung cancer findings are unlikely to be due to smoking because other smoking-related cancers, such as those of the bladder, esophagus, and buccal cavity, showed no exposure–response relationship with chlorpyrifos use after accounting for cigarette smoking, whether adjusted by pack-years, number of packs smoked per day, or years smoked (data not shown). Furthermore, neither index of chlorpyrifos exposure (lifetime exposure-days or intensity-weighted exposure-days) was correlated with pack-years smoked ($r = .02$ and .03, respectively), so confounding by smoking is not likely to be an important issue in our study.

Previous studies have reported an increased risk of rectal cancer among pesticide-exposed workers *(35)* and farmers *(36,37)*. We found that the risk of rectal cancer increased with chlorpyrifos exposure; the trend was statistically significant for lifetime exposure-days but only approached statistical significance for intensity-weighted exposure-days. For both exposure measures, however, the positive trend was due to the elevated risk in the highest exposure category. The small numbers of cases and the non-monotonic shape of the exposure–response curve therefore limit our conclusions about rectal cancer.

The etiology of brain cancer is poorly understood, but brain cancer has been linked to agricultural exposure, including exposure to pesticides *(38)*. We found a statistically significantly increased risk of brain cancer with intensity-weighted exposure-days but not with lifetime exposure-days, although there were too few cases to produce a definitive answer. Because chlorpyrifos has neurotoxic effects in rat brains *(39)*, the association of chlorpyrifos with brain cancer should be further studied in the cohort as additional cases occur.

We found a two-fold increased risk for lymphohematopoietic cancers in the highest category of intensity-weighted exposure-days but not in any other category of exposure. However, the lack of a corresponding increase with lifetime exposure-days is difficult to explain and weakens the argument for a causal relation.

The Agricultural Health Study has several important strengths. It is the largest epidemiologic study of pesticide applicators exposed to chlorpyrifos that has been conducted to date. Although the duration of cohort follow-up is still relatively short (6.4 years on average), as of December 2001, this analysis had 90% statistical power to detect a 1.5-fold increase in lung cancer incidence. All exposure information was collected before the diagnosis of cancer, which avoids case–recall bias. This study included comprehensive questionnaire data that were used to quantitatively estimate chlorpyrifos exposure levels and to control for potential confounding from lifestyle or other occupational exposures. The association between chlorpyrifos and lung cancer was observed whether low exposed or non-exposed persons were used as a referent group.

A limitation of this study and of almost all studies of pesticide users is that people who apply pesticides are seldom exposed to just a single agent. Coble et al. *(40)* evaluated the relationships among different agricultural exposures in this cohort and found that substantial bias due to unrecognized confounding from exposure to multiple agents was unlikely. To reduce the possibility of residual confounding, we adjusted the lung cancer risk estimates by including in our models the four pesticides whose use is most highly correlated with the use of chlorpyrifos. We further mitigated the possibility of uncontrolled confounding by using the pesticide applicators with the lowest exposure, instead of nonexposed applicators, as a reference group. Results were similar with both reference groups, suggesting that uncontrolled confounding was unlikely. Total years of pesticide application (of any pesticide) is a good measure of occupational exposure experienced by pesticide applicators, both farmers and commercial applicators, and therefore an excellent surrogate for many other factors. This variable was not statistically significant in the multivariable analysis, and it did not have a statistically significant effect on the risk estimates that were associated with chlorpyrifos exposure.

Another possible limitation is that the formulation, work practices, and application methods associated with chlorpyrifos use may have changed over the years. These changes may result in some exposure misclassification, particularly in studies in which exposure is based on subject's recall. However, recall of pesticide use by the Agricultural Health Study cohort has been shown to be as reliable as recall of other factors routinely evaluated by questionnaire in epidemiology studies, such as smoking and alcohol use, and to be better than recall of other factors, such as consumption of fruits and vegetables and physical activity *(41)*. Hoppin et al. *(42)* also demonstrated that participants in our cohort provided plausible information regarding the duration of use of specific pesticides.

A potential limitation in chlorpyrifos exposure classification also should be considered. Among the chlorpyrifos-exposed applicators, most (93%) were farmers, and 7% were full-time applicators. The exposed group used chlorpyrifos for 6.6 years on average and for 9.4 days per year on average. Although the highest quartile of exposed applicators had more than 56 lifetime exposure-days, applicators in this quartile had much higher lifetime exposure-days on average (i.e., 224 mean lifetime exposure-days and 116 median lifetime exposure-days). We defined pesticide applicators who used chlorpyrifos for agricultural purposes as an exposed group and applicators who did not use chlorpyrifos as a nonexposed group. However, chlorpyrifos is a widely used insecticide for agricultural and nonagricultural purposes in the United States. Therefore, both groups may also be exposed to chlorpyrifos by nonoccupational routes to some extent, causing potential nondifferential misclassification of exposure.

Skin exposure is believed to be the main route of pesticide absorption into the body among applicators in agriculture *(43)*. Therefore, the intensity level algorithm used to estimate pesticide exposure in this study emphasizes dermal absorption *(24)*. However, for lung cancer, the respiratory route may be more important, and intensity-weighted exposure-days

may be less appropriate than lifetime exposure-days. In this lung cancer analysis, however, results were similar with (intensity-weighted exposure-days) and without (lifetime exposure-days) taking into account factor weights associated with dermal exposure.

A total of 955 (less than 1%) of the cohort members left the states of Iowa and North Carolina during the period of the study (i.e., 1993–2001), and any incident cancers among this group are lost to the state cancer registries. This small portion of the total cohort is younger and more educated, smokes less, and has a slightly lower frequency of family history of cancer than the total cohort (data not shown) and is therefore likely to generate proportionally fewer cancers than the rest of the cohort. To assess the magnitude of the potential bias caused by including this group with a low cancer risk in the analysis, we recalculated the lung cancer risk estimates by adding all the lost person-years generated by this group to the denominator and assuming no cancer cases in the numerator. We observed only minimal changes in the risk estimates (data not shown), and these did not affect our conclusions.

In summary, our findings suggest an association between chlorpyrifos use and incidence of lung cancer among applicators in the Agricultural Health Study. The increased risk of lung cancer with increasing chlorpyrifos use was consistent after controlling for state of residence and for a variety of lifestyle factors, including smoking, other occupational exposures, previous lung diseases, type of farm, and vegetable intake. However, lung cancer was not an *a priori* hypothesized site linked to chlorpyrifos exposure, and thus our results must be interpreted cautiously, pending confirmatory studies in the Agricultural Health Study and elsewhere.

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NOTES

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